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FINITE ELEMENT ANALYSES OF VIBRATION RESPONSES OF AN OSTEOBLAST IN VITRO

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Introduction: Mechanical stimulation to bones affects bone formation and bone cells have been reported to sense and respond to mechanical stimulation. However, osteoblast responses to mechanical stimulation are less understood. By using a finite element (FE) method, the aim of this study was to investigate the responses of a cultured osteoblast to vibration of a broad range of frequencies. Firstly, the mode shapes were developed and natural frequencies were extracted. Subsequently, the base excitation acceleration 1 g and vibration frequency range 1–80 Hz were used to conduct harmonic analyses. Finally, the response of harmonic vibration was obtained as the curve of displacement versus frequency.

Subjects and Methods: According to the images of osteoblasts in vitro, an idealized FE model of an osteoblast was created using FE software ABAQUS. In this study, the osteoblast was comprised of three components, namely cell membrane, cytoplasm and nucleus. Based on parameters from previous studies, the whole volume of the osteoblast and nucleus volume were $\sim 3000 \mu\text{m}^3$ and $105 \mu\text{m}^3$, respectively. In addition, the cell height was $\sim 9.4 \mu\text{m}$ and the membrane thickness was $\sim 6 \text{ nm}$. The cell was assumed as an elastic isotropic material. The natural frequencies were extracted by using ABAQUS software. The governing dynamic response equation of vibration of the osteoblast may be expressed by Eq (1): $M\ddot{u} + C\dot{u} + Ku = F$ where M , C and K are the mass matrix, the damping coefficient matrix and the spring stiffness matrix in the system, respectively. F is the loading, and u , \dot{u} and \ddot{u} are the acceleration, the velocity and the displacement vector, respectively. Also, one degree-of-freedom vibration system was considered. Additionally, the base excitation was applied with 1 g and frequency range between 1 and 80 Hz to carry out the harmonic analysis.

Results: The natural frequencies were extracted for the first ten modes of the FE model. They were between 21.40 Hz and 40.96 Hz. In addition, the relationship between displacement and frequency was obtained for the harmonic vibration, and the mode shapes of the FE model at peak frequency for acceleration in the X-direction, Y-direction and Z-direction were determined.

Discussion and Conclusion: In this current study, an osteoblast FE model was developed to conduct the vibration analyses of a cultured osteoblast in vitro. The natural frequencies were obtained and the range is between 21.40 Hz and 40.96 Hz. This range of natural frequency accords with those of previous studies. The results of harmonic analysis showed that the peak value of the displacement of the cell's center point occurred at nearly the same natural frequencies for the X-direction and Y-direction, whilst the peak value of the displacement center point of the cell occurred at the fourth mode natural frequency for the Z-direction. This is because the shape of the cultured osteoblast is different in three directions. Results from this study can provide a guide and reference for the vibration experiments of osteoblasts in vitro and other related investigations of bone cells.

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EFFECT OF LOW DOSE IRRADIATION ON TITANIUM PARTICLES WITH LPS ADHERED ACTIVATED MACROPHAGE FUNCTION AND SIGNALING PATHWAYS MAY BE INVOLVED

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Objective: To study if low dose irradiation (LDI) can inhibit the macrophage inflammatory reaction, which stimulated by the Ti particles with LPS adhered. To study signaling pathways may be involved.

Methods: Place all cultured cells were divided into three groups: control group (Ti-negative group), non-irradiated group (0 Gy group) and the irradiation group (0.5 Gy group). 0 Gy group and 0.5 Gy group stimulated by of Ti particles with LPS adhered. 0.5 Gy group received 0.5 Gy irradiation 1h after stimulation. The expression of IL-1 β , IL-6 and TNF- α gene were measured by Real-time PCR method at 2,4,8,12,24h after stimulation. The concentration of IL-1 β , IL-6 and TNF- α in supernatant were measured by ELISA at 12,24h after stimulation. The expression of Ph-p65, p65, Ph-perk, Ph-p38 in three groups were measured by Western Blot method at 4,8,12h after irradiation.

Results: Real-time PCR showed 0.5 Gy of LDI can reduce the increased expression of IL-1 β , IL-6 and TNF- α in macrophage by stimulated by LPS Ti particles with LPS adhered. ELISA analysis showed 0.5 Gy of LDI could also inhibit the increased secretion of IL-1 β , IL-6 and TNF- α . This inhibition is more obvious 12h after irradiation. The secretion of the most sensitive factor TNF- α is barely increased between 12 ~ 24h. Western Blot analysis showed 0.5 Gy of LDI can reduce the phosphorylation of p65 and ERK after, but had no effect on the phosphorylation of p38.

Conclusion: After playing a biological role, LDI not only effectively inhibited the inflammatory stimuli of the primary mouse peritoneal macrophage, which caused by Ti particles with LPS adhered, but also significantly reduce the formation of IL-1 β , IL-6, TNF- α . This effect may be achieved by inhibition two pathways of NF- κ B and ERK.

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EFFECT OF CYCLIC TENSILE STRESS DURATION ON RABBIT ANNULUS FIBROUS-DERIVED STEM CELLS

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Background: Mechanical forces play a critical role in the degeneration of intervertebral disc, especially in annulus fibrosus (AF), which is a leading cause of low back pain. As the mechanical forces on AF tissue are undergone by the resident cells, in this study we applied different durations of cyclic tensile stress to rabbit annulus fibrosus-derived stem cells (AFSCs) and explored the effect of mechanical stimulation duration on the matrix metabolisms of cells.

Methods: Rabbit AFSCs were isolated using enzymatic hydrolysis and subsequently seeded on an elastic silicone dish coated with fibronectin. The cells were then subjected to cyclic tensile stress (CTS) at a frequency of 0.5 Hz with 5% strain magnitudes for various durations. Changes in gene expression and related proteins were determined using RT-qPCR and Elisa analyses. The morphology of cells was visualized by cytoskeleton staining. The cell traction forces (CTFs) of cells were measured using cell traction force microscopy (CTFM).

Results: The expression of anabolic genes (type I collagen, type II collagen, aggrecan) and related proteins in AFSCs undergoing moderate mechanical stimulation duration (4 h) increased after CTS. The expression of catabolic genes (MMP-3, MMP-13, TIMP-1) and related proteins in AFSCs undergoing excessive mechanical stimulation duration (16 h) increased, yet the expression of anabolic genes (aggrecan, type I collagen, and type II collagen) and related proteins decreased. There were no obvious difference in cell morphology in the 0, 2, 4 and 8 h stimulation groups. CTFM measurements showed that the CTF of AFSCs gradually decreased with the increase of CTS duration.

Discussion and Conclusion: The expression of anabolic genes and related proteins increased under moderate mechanical stimulation duration. On the other hand, the expression of catabolic genes and related proteins increased under excessive mechanical stimulation duration, yet the expression of anabolic genes and related proteins decreased. These findings may help understand the mechanism of AF